

What is Claimed:

1. A method of identifying hairpin nucleic acid probes, the method comprising:
 - providing a target nucleic acid sequence that is larger than about 100 nucleotides in length;
 - 5 predicting a folded structure of the target nucleic acid sequence;
 - identifying a nucleotide sequence of a hairpin within the folded structure of the target nucleic acid sequence; and
 - 10 predicting a folded structure for the identified nucleotide sequence of the hairpin, in the absence of other nucleotides of the target nucleic acid sequence, wherein the folded structure of the hairpin has a predicted E value of at most about - 3 kcal/mol.
- 15 2. The method according to claim 1 wherein the nucleotide sequence of the hairpin is between about 12 and about 60 nucleotides in length.
3. The method according to claim 1 wherein the folded structure of the hairpin has a predicted E value of between about - 4 kcal/mol and about - 12 kcal/mol.
- 20 4. The method according to claim 1 further comprising:
 - predicting a folded structure of a duplex formed between the hairpin and its complement.
- 25 5. The method according to claim 4 further comprising:
 - determining whether duplex formation is energetically favorable.
- 30 6. The method according to claim 1 further comprising:
 - performing a database search for nucleotide sequences that are similar to the identified nucleotide sequence of the hairpin.

7. The method according to claim 6 further comprising:
determining, from the results of the performed database
search, whether a clear demarcation exists between scores for target nucleic acid
sequences and scores for non-target nucleic acid sequences.

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8. The method of preparing a molecular beacon comprising:
providing a hairpin nucleic acid probe identified according to
the method of claim 1; and
tethering a fluorescent label and a quenching agent to the
10 opposed termini of the provided hairpin nucleic acid probe to form a molecular
beacon,
wherein the molecular beacon is substantially non-fluorescent
in the absence of a nucleic acid complementary to the hairpin nucleic acid probe.

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9. The method according to claim 8, wherein said providing
comprises:
synthesizing a nucleic acid molecule corresponding to the
nucleotide sequence of the hairpin probe.

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10. The method according to claim 8, wherein the fluorescent
label is tethered to the 5' terminus and the quenching agent is tethered to the 3'
terminus.

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11. The method according to claim 8, wherein the fluorescent
label is tethered to the 3' terminus and the quenching agent is tethered to the 5'
terminus.

12. The method according to claim 8, wherein the quenching
agent is a solid surface.

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13. The method according to claim 8, wherein the quenching
agent is a micro- or nano-particle.

14. The method according to claim 8, wherein the fluorescent label is a fluorescent dye, semiconductor quantum dot, lanthanide atom-containing complex, or fluorescent protein.

5 15. The method according to claim 8, wherein the quenching agent is a metal or 4-([4-(Dimethylamino)phenyl]azo)benzoic acid.

10 16. The method according to claim 15, wherein the metal is gold, silver, platinum, copper, cobalt, iron, or iron-platinum.

15 17. A method of preparing a hairpin nucleic acid molecule comprising:

synthesizing a hairpin nucleic acid molecule identified according to the method of claim 1.

18. An isolated nucleic acid molecule prepared according to the method of claim 17.

19. An isolated molecular beacon comprising:
20 the nucleic acid molecule according to claim 18;
a fluorescent label tethered to one terminus of the nucleic acid molecule; and
a quenching agent tethered to the other terminus of the nucleic acid molecule.

25 20. The isolated molecular beacon according to claim 19, wherein the fluorescent label is tethered to the 5' terminus and the quenching agent is tethered to the 3' terminus.

30 21. The isolated molecular beacon according to claim 19, wherein the fluorescent label is tethered to the 3' terminus and the quenching agent is tethered to the 5' terminus.

35 22. The isolated molecular beacon according to claim 19, wherein the quenching agent is a solid surface.

23. The isolated molecular beacon according to claim 19, wherein the quenching agent is a micro- or nano-particle.

24. The isolated molecular beacon according to claim 19, wherein 5 the fluorescent label is a fluorescent dye, semiconductor quantum dot, lanthanide atom-containing complex, or fluorescent protein.

25. The isolated molecular beacon according to claim 19, wherein the quenching agent is a metal or 4-([4-(Dimethylamino)phenyl]azo)benzoic acid.

10 26. The isolated molecular beacon according to claim 19, wherein the metal is gold, silver, platinum, copper, cobalt, iron, or iron-platinum.

27. The isolated molecular beacon according to claim 19, wherein 15 the nucleic acid molecule is characterized by a predicted E value of at most about -3 kcal/mol.

28. The isolated molecular beacon according to claim 19, wherein the predicted E value is between about -4 kcal/mol and about -12 kcal/mol.

20 29. The isolated molecular beacon according to claim 19, wherein the nucleic acid molecule is between about 12 and about 60 nucleotides in length.

30. The isolated molecular beacon according to claim 19, wherein 25 hybridization between the nucleic acid molecule and its perfect complement is predicted to have a lowest free energy value that is at least about a two-fold increase over the lowest predicted energy value of the nucleic acid molecule alone.